

In re Application of: Evans et al.
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PATENT
Attorney Docket No.: SALK1470-2
(088802-1852)

16. (Twice amended) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said receptor is introduced into said cells by a receptor expression vector comprising a DNA segment encoding PPAR- γ , and

wherein said reporter vector comprises:

- Sub H2
- (a) a promoter that is operable in said cell,
 - (b) a hormone response element, and
 - (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

Please add new claims 29-35 as follows:

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29. (New) A method of testing a compound for its ability to regulate transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR- γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said cells express native PPAR- γ , and

wherein said reporter vector comprises:

- Sub H57
- (a) a promoter that is operable in said cell,
 - (b) a hormone response element, and
 - (c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof,

G2

wherein an increase or decrease in the level of the reporter protein detected when said cells are contacted with said compound, relative to the level of the reporter protein detected when said cells are not contacted with said compound, is indicative of a compound that regulates the transcription-activating effects of said receptor.

30. (New) A method according to claim 29, wherein said hormone response element is a direct repeat of two or more half sites separated by a spacer of one nucleotide, wherein said spacer can be A, C, G or T, wherein each half site comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-; and

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wherein said response element is optionally preceded by N_x , wherein x falls in the range of 0 up to 5.

31. (New) A method according to claim 30, wherein said response element has at least one copy of the minimal sequence:

AGGACA A AGGTCA (SEQ. ID NO. 5),

wherein said minimal sequence is optionally flanked by additional residues.

32. (New) A method according to claim 30, wherein said response element has at least one copy of the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ. ID NO. 6).

33. (New) A method according to claim 29, wherein said compound is a putative antagonist for said peroxisome proliferator activated receptor-gamma, and wherein said contacting is carried out in the presence of

increasing concentrations of said compound, and

a fixed concentration of at least one agonist for said peroxisome proliferator activated receptor-gamma,

wherein a decrease in the level of the reporter protein detected when said cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when said cells are contacted with said agonist alone, is indicative of a compound that is an antagonist of said receptor.

34. (New) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR- γ agonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said agonist, relative to the level of the reporter protein detected when cells are contacted with said agonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.

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Sub H67
cont.
G/2
Ant

35. (New) A method according to claim 29, wherein said contacting is carried out in the further presence of at least one PPAR- γ antagonist,

wherein an increase or decrease in the level of the reporter protein detected when cells are contacted with said compound and said antagonist, relative to the level of the reporter protein detected when cells are contacted with said antagonist alone, is indicative of a compound that regulates the transcription-activating effects of said receptor.
